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Antibody-Mediated In Vitro Neutralization of Human Immunodeficiency Virus Type 1 Abolishes Infectivity for Chimpanzees

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This study was undertaken to establish whether antibody directed against the human immunodeficiency virus type 1 (HIV-1) principal gp120 type-specific neutralization determinant can abolish the infectivity of HIV-1 in chimpanzees. Challenge inocula of the IIIb virus isolate were mixed in vitro with either immunoglobulin G (IgG) from an uninfected chimpanzee, nonneutralizing IgG from an HIV-seropositive human, a virus-neutralizing murine monoclonal antibody directed against the HIV-1 IIIb isolate, or virus-neutralizing IgG from a chimpanzee infected with the IIIb isolate. Both neutralizing antibodies were directed against the principal neutralization determinant of the challenge isolate. Establishment of infection following inoculation of each virus-antibody mixture into chimpanzees was assessed by virus-specific antibody development and by virus isolation. No protective effect was noted either with the control IgG or with the nonneutralizing anti-HIV IgG. By contrast, the polyclonal chimpanzee virus-neutralizing IgG prevented HIV-1 in vivo infection, while the neutralizing monoclonal antibody notably decreased the infectivity of the challenge virus. Hence, antibody to the gp120 principal neutralization determinant is able both to prevent HIV-1 infection in vitro and to inhibit infection in vivo.

The human immunodeficiency virus type 1 (HIV-1), a member of the *Lentivirinae* subfamily of retroviruses, is the causative agent of the acquired immunodeficiency syndrome. The virion surface is composed largely of a 120-kilodalton (kDa) glycoprotein, gp120, which is noncovalently bound to a 41-kDa anchor glycoprotein. Both glycoproteins derive by cleavage from a 160-kDa precursor. Previous studies have demonstrated that gp120-related immunogens, whether purified from virus-infected cells or expressed by several recombinant systems, commonly elicit a type-specific in vitro virus-neutralizing antibody response in various test animals (2, 3, 8, 11, 15, 18, 21, 23, 25). The structural determinant to which this virus-neutralizing antibody binds encompasses amino acid residues 296 to 331 of gp120 (IIIb isolate) and is termed the principal neutralization determinant (PND) (5–7, 9, 10, 24). This region represents the glycoprotein's third hypervariable domain (V3). Synthetic peptide fragments of this domain induce virus-neutralizing antibody (7, 10, 19), and a number of monoclonal antibodies have been derived which bind to this site and effectively neutralize virus infectivity in vitro (4, 14, 27, 28).

This study was undertaken to establish whether antibody directed against the PND can abolish the infectivity of HIV-1 for chimpanzees. Previously reported studies of actively or passively immunized chimpanzees challenged with HIV-1 have failed to address this issue directly. None of the animals in the active immunization experiments reported to date had circulating virus-neutralizing antibody titers at the time of challenge (1, 3, 8), and while the passive immunization study reported by Prince et al. (20) did establish significant levels of circulating neutralizing antibody before virus challenge, the passively administered HIV-1 immune globulin was uncharacterized with respect to anti-PND antibody specific for the challenge virus isolate.

The design of the present study was experimentally straightforward. Virus and appropriately specific anti-PND antibody were mixed in vitro under conditions leading to complete in vitro neutralization of the virus. The virus-antibody mixture was then inoculated into a susceptible chimpanzee. Including controls, four purified immunoglobulin G (IgG) samples were tested: (i) nonneutralizing control chimpanzee IgG, (ii) nonneutralizing anti-HIV-1 polyclonal human IgG, (iii) a neutralizing murine monoclonal antibody against the PND, and (iv) neutralizing polyclonal chimpanzee IgG directed to the PND.

MATERIALS AND METHODS

Animals, virus, and antibodies. The study used four adult chimpanzees (*Pan troglodytes*). The animals were maintained under conditions exceeding the requirements specified by the National Institutes of Health Guide for the Care of Laboratory Animals. Inoculations and bleedings were performed after tranquilization with ketamine hydrochloride at 15.0 mg/kg.

The challenge virus stock of HIV-1 (IIIb isolate) was generously provided by Larry Arthur and Peter Fischinger (National Cancer Institute). This stock has been titrated previously in chimpanzees (1).

The HIV-seropositive human and the two chimpanzee IgG

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TABLE 1. IgG preparation characteristics

Preparation	IgG sample	Anti-HIV-1 reactivity[a]	Neutralizing activity[b]	Binding to PND[c]
I	Seronegative chimpanzee x1	−	<1:10	−
II	Seropositive human (sample 498)	+	<1:10	−
III	0.5β monoclonal antibody	NT[d]	1:5,120	+
IV	Seropositive chimpanzee x118	+	1:640	+

[a] Anti-HIV-1 activity was measured by commercial Western blot (Du Pont, Wilmington, Del.). Symbols: +, positive binding to all virus-specific protein bands; −, no binding to any of the virus-specific proteins.

[b] Neutralizing activity was measured against the IIIb isolate of HIV-1 as described in Materials and Methods. The values represent the highest dilution of the purified antibody preparation which completely neutralized the infecting virus. The quantity of virus used in this assay was identical to the quantity used for chimpanzee challenge (see text).

[c] Binding was measured by ELISA with a synthetic peptide fragment (RP135 [24]) of the IIIb isolate PND as the substrate. Symbols: +, positive ELISA signal (≥10-fold above background) with an undiluted sample of the antibody preparation; −, background ELISA reading with an undiluted sample.

[d] NT, Not tested.

samples were prepared from plasma by protein A-affinity chromatography. The monoclonal antibody was purified from mouse ascites fluid by the same method.

In vitro neutralization and chimpanzee infection. The challenge virus was diluted 1:25 in physiologic saline prior to use. Cell culture titration of the virus at the time of use yielded an infectious-dose value equivalent to approximately 140 chimpanzee 50% infectious doses per ml as defined by Arthur et al. (1). Each purified antibody preparation (1 ml) was added to one of four identical 1.0-ml portions of virus. The two virus-neutralizing antibody preparations were mixed with the virus inoculum well in excess of the minimum levels needed for complete virus neutralization (Table 1). The antibody-virus mixtures were incubated at room temperature (19 to 25°C) for 90 min. Each mixture was then inoculated intravenously into one of four HIV-seronegative chimpanzees. The animals were tested for HIV-1 infection by virus isolation. Isolation results were confirmed by polymerase chain reaction (PCR)-mediated amplification of virus-specific DNA sequences from peripheral blood mononuclear cells (PBMC). The development of specific antibody responses was also assessed.

Virus neutralization assay. The assay was performed as described by Robertson et al. (22). All test sera were heat-inactivated at 56°C for 60 min before use. Briefly, a titrated amount of HIV-1 stock was added to 1:2 serial dilutions of the test serum. Following a 60-min incubation at room temperature, 10^5 MT-4 human lymphoid cells were added to each virus-serum sample. The cells then were incubated at 37°C for 7 days. Virus-mediated cell killing was assessed at this time by staining of the cell cultures with a tetrazolium dye. Virus neutralization was manifested by the prevention of virus-induced cell death. Results were confirmed by observation of the MT-4 cells with virus-specific immunofluorescence.

Detection of virus-specific DNA sequences. PBMC were obtained from the test chimpanzees by Ficoll gradient fractionation of heparinized blood and were processed in parallel with PBMC from three uninfected chimpanzees. High-molecular-weight DNA was isolated from the cells by sodium dodecyl sulfate-proteinase K digestion and phenol-chloroform extraction as described before (13). DNA samples were digested with RNase A, reextracted, and concentrated by ethanol precipitation and centrifugation. The samples were then suspended in a buffer containing 10 mM Tris (pH 8.0), 25 mM NaCl, and 0.1 mM EDTA and adjusted to a concentration of 35 μg/ml. PCR amplification was performed with the DNA thermal cycler and the GeneAmp kit of Perkin-Elmer Cetus (Norwalk, Conn.). Two sets of oligonucleotide primers flanking the HIV-1 PND (set 1 at positions 7065 to 7088 and 7366 to 7391 and set 2, nested within primer set 1, at positions 7096 to 7141 and 7282 to 7312 of the HXB2 genome; GenBank accession no. K03455) were prepared by the Synthecell Corporation (Rockville, Md.). Thirty PCR cycles, consisting of denaturation at 94°C for 1.5 min, hybridization at 55°C for 1.5 min, and polymerization at 72°C for 1.5 min were used to amplify 1.0 μg of DNA with 100 pmol of the set 1 primers. Then, 5 μl of this reaction was reamplified under the same PCR conditions with 100 pmol of the set 2 primers.

RESULTS

Four purified IgG preparations were tested for their ability both to neutralize HIV-1 in vitro and to abolish viral infectivity in vivo. The characteristics of each preparation are summarized in Table 1.

The preparation I sample was a control IgG purified from the plasma of an uninfected chimpanzee (x1).

Preparation II was IgG purified from the plasma of an HIV-1-seropositive human (sample 498). At the dilution used in this study, the purified IgG had no neutralizing activity directed against the challenge IIIb isolate and no binding activity directed against the isolate's PND. However, the IgG was positive for activity directed against all of the viral proteins as determined by commercial Western immunoblot with a viral lysate. This IgG was included in the study to establish whether nonneutralizing anti-HIV-1 antibody exhibits any in vivo protective effect.

Preparation III was a purified murine monoclonal antibody, 0.5β. This antibody was elicited by the viral gp120 glycoprotein, is IIIb isolate specific in its neutralizing activity, and is directed against the IIIb isolate's PND (14).

Preparation IV was a polyclonal IIIb isolate-specific neutralizing IgG which had been purified from the plasma of a previously infected chimpanzee (x118). This animal had

TABLE 2. Absorption of preparation IV IgG virus-neutralizing activity by a PND-specific peptide[a]

Sample	Column substrate[b]	RP135 ELISA titer[c]	Neutralizing antibody titer[d]
Preabsorption	RP135	1:5,120	1:640
Postabsorption	RP135	<1:10	<1:10
Preabsorption	Mock	1:5,120	1:640
Postabsorption	Mock	1:5,120	1:640

[a] Absorption was performed with an affinity column of synthetic peptide covalently linked to Sepharose CL-4B. The antibody was loaded onto the column in phosphate-buffered saline, and the flowthrough was tested for antipeptide and virus-neutralizing activities.

[b] RP135 is a synthetic peptide representative of the IIIb isolate PND (24). The mock column contained unconjugated resin.

[c] Anti-RP135 titers were determined by using a standard ELISA. Free RP135 peptide was used as the substrate. The values represent the highest dilution of column flowthrough which yielded an ELISA signal 2.1-fold above the control background.

[d] Neutralizing activity was measured against the IIIb isolate of HIV-1 as described in Materials and Methods. The values represent the highest dilution of the column flowthrough which completely neutralized the infecting virus.

TABLE 3. Chimpanzee p.i. serological and virus isolation profiles

Section (chimpanzee no.)	Inoculum	Time p.i. (wk)	HIV-1[a] ELISA titer	HIV-1[a] Western blot	Neutralizing antibody[b]	Virus isolation[c]
A (x222)	Virus + prepn I	0	<1:20	−	<1:10	ND[d]
		2	<1:20	−	<1:10	ND
		4	<1:20	−	<1:10	ND
		6	1:40	+/−	<1:10	ND
		8	1:20	+/−	<1:10	ND
		10	1:640	+	1:20	ND
		12	1:1,280	+	1:160	+
		14	1:2,560	+	1:160	+
		16	1:5,120	+	1:320	ND
		17	1:5,120	+	1:320	+
		19	1:10,240	+	1:320	+
		21	1:10,240	+	1:320	+
		23	1:10,240	+	1:320	+
		25	1:5,120	+	1:320	ND
		27	1:5,120	+	1:320	+
		31	1:10,240	+	1:640	+
		36	1:5,120	+	1:640	ND
		42	1:5,120	+	1:640	+
		67	1:5,120	+	1:640	+
		76	1:5,120	+	1:1,280	+
B (x177)[e]	Virus + prepn II	0	<1:20	−	<1:10	ND
		2	<1:20	−	<1:10	ND
		4	1:20	−	<1:10	ND
		6	1:160	+/−	<1:10	ND
		8	1:320	+/−	<1:10	ND
		10	1:160	+	1:10	ND
		12	1:320	+	1:10	+
		14	1:320	+	1:40	+
		16	1:160	+	1:40	ND
		17	1:640	+	1:40	+
		19	1:640	+	1:40	+
		21	1:1,280	+	1:80	+
		23	1:640	+	1:80	+
		25	1:1,280	+	1:80	ND
		27	1:1,280	+	1:80	+
		31	1:5,120	+	1:160	+
		36	1:2,560	+	1:320	ND
		42	1:12,560	+	1:320	+
C (×)	Virus + prepn III	0	<1:20	−	<1:10	ND
		2	<1:20	−	ND	ND
		4	<1:20	−	ND	ND
		6	<1:20	−	ND	ND
		8	<1:20	−	ND	ND
		10	<1:20	−	ND	ND
		12	<1:20	+/−	ND	+
		14	1:20	+/−	ND	−
		16	1:20	+/−	ND	ND
		17	1:20	+/−	ND	−
		19	1:40	+/−	ND	−
		21	1:40	+/−	ND	+
		23	1:80	+/−	<1:10	−
		25	1:80	+/−	ND	ND
		27	1:80	+/−	<1:10	−
		31	1:80	+/−	<1:10	−
		36	1:160	+/−	<1:10	ND
		42	1:160	+	<1:10	−
		67	1:1,5120	+	1:160	+
		76	1:15,120	+	1:160	+
D (×81)	Virus + prepn IV	0	<1:20	−	<1:10	ND
		2	ND	−	ND	ND
		4	ND	−	ND	ND
		6	ND	−	ND	ND
		8	ND	−	ND	ND
		10	ND	−	ND	ND
		12	ND	−	ND	−
		14	ND	−	ND	−
		16	ND	−	ND	ND
		17	ND	−	ND	−
		19	ND	−	ND	−
		21	ND	−	ND	−
		23	ND	−	<1:10	−
		25	ND	−	ND	ND
		27	<1:20	−	<1:10	ND
		31	ND	−	ND	−
		36	<1:20	−	ND	ND
		42	<1:20	−	<1:10	−
		67	<1:20	−	<1:10	−
		76	<1:20	−	<1:10	−

[a] HIV-1 ELISA was performed with a commercial assay kit. Titers were calculated according to the manufacturer's instructions. HIV-1 viral lysate Western blot was performed with a commercial kit. The sera were tested at a 1:10 dilution. Symbols: +, reactivity noted with all of the virus-specific protein bands; +/−, reactivity noted with four or fewer virus proteins; −, no reactivity against any of the virus proteins.

[b] Neutralizing antibody was determined against the IIIb isolate of HIV-1 as described in Materials and Methods. The values represent the highest serum dilution which completely neutralized the assay's infecting virus.

[c] Virus isolation from peripheral blood mononuclear cells (PBMC) was performed by in vitro co-cultivation with uninfected human PBMC as described by Nara et al. (16).

[d] ND, Not done.

[e] Chimpanzee x177 died before the week 67 sample was obtained. Death was due to causes unrelated to this study.

been inoculated 1 year earlier with the HIV-1 IIIb isolate. Plasma from x118 was obtained with the kind permission of H. J. Alter (National Institutes of Health). Absorption studies with a synthetic peptide (RP135 [24]) representative of the IIIb isolate PND showed that the IgG's entire neutralizing capacity was directed against this determinant. Absorption onto an RP135-containing resin led to complete loss of in vitro neutralizing activity (Table 2).

Each IgG sample was mixed with a sample of the challenge virus and incubated at room temperature for 90 min. Then, 1/40th of each mixture was withdrawn and analyzed for its in vitro infectivity with the highly HIV-susceptible MT-4 lymphoid cell line. The virus inocula mixed with the nonneutralizing IgG preparations I and II both proved infectious, yielding virus-specific immunofluorescence several days after inoculation onto MT-4 cells. In contrast, no virus-specific infection was observed for 8 weeks following cell culture inoculation of virus mixed with neutralizing IgG preparations III and IV.

The remainder of each virus-antibody mixture was injected intravenously into one of four HIV-seronegative chimpanzees. Each animal was then assessed as described in Materials and Methods for the development of persistent infection. The results are presented in Table 3.

The animal inoculated with the virus-preparation I control IgG mixture (chimpanzee x222) exhibited initial immunological signs of HIV-1 infection by 6 weeks postinoculation

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